155. The method of claim 106, wherein said medium is serum-free, and wherein said growth is high-density growth.

156. The method of claim 112, wherein said growth is high-density growth.

Sub B2 (157) A method for replacing protein in a mammalian cell culture medium, said method comprising

Cancel

replacing insulin with a Zn^{2+} salt and/or replacing transferrin with a Fe^{2+} chelate and/or replacing transferrin with a Fe^{3+} chelate.

Sub E10 (158) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a serum-free, non-animal derived cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension.--

add E11

Remarks

Applicants respectfully request that the Examiner reconsider and withdraw the outstanding rejections.

I. Status of the Claims

Claims 1, 15, 22, 106, 146, 152 and 153 have been amended. Claims 78, 83, 141 and 142 have been canceled without prejudice to, or disclaimer of, the subject matter therein.

Claims 154-158 have been added. Claims 1-37, 73-77, 79-82, 106-112, 140, 143 and ~~145~~-158 are active in the present application.

Claims 140-153 were added in the Second Preliminary Amendment and Reply to Restriction Requirement filed on September 24, 1999. Applicants thank the examiner for examining claims 140-153 with elected claims 1-37, 73-83 and 106-112.

II. Support for the Amendment

Support for the amendment of claims 1, 15 and 22 is found in the specification, for example, at page 1, line 6; page 12, line 9; and page 26, lines 12-16.

Support for the amendment of claim 106 is found in the specification at page 12, lines 12-13; page 19, line 6; page 30, lines 18-19; and page 40, lines 8-9.

Claim 141 has been canceled because it was duplicative of claim 6.

Support for the amendment of claims 152 and 153 is found in the specification at page 40, line19 to page 41, line 3.

Support for new claim 154 is found in the specification at page 1, line 5.

Support for new claim 155 is found in the specification at page 1, line 5; and page 26, lines 17-18.

Support for new claim 156 is found in the specification at page 26, lines 17-18.

Support for new claim 157 is found in the specification at page 13, lines 4-8; and page 19, lines 4-6.

Support for new claim 158 is found in the specification, for example, at page 1, line 5; page 12, line 24 to page 13, line3; and page 21, lines 20-21.

No new matter has been added by these amendments.

III. Information Disclosure Statement

The First Supplemental Information Disclosure Statement filed September 24, 1999 contains an inadvertent error. At page 10, in section VI, "Additional Information," Applicants wrote:

> In March, 1996, Dr. Gorfien attended a talk by Dr. Michael Shuler, a researcher at Cornell University. In his talk, Dr. Shuler discussed the use of dextran sulfate to culture insect cells. Prior to the talk by Dr. Shuler, Dr. Gorfien and Dr. Epstein had discussed Dr. Epstein's use of dextran sulfate in the suspension culture medium. After the talk by Dr. Shuler, Dr. Gorfien had the idea to use dextran sulfate in the CHO III and CD CHO media.

Mr. Glenn Godwin is co-inventor in the present application. It was Mr. Godwin, not Dr. Gorfien, who attended the talk by Dr. Shuler in March, 1996. Dr. Shuler presented that talk at the March, 1996 American Chemical Society Meeting in New Orleans, Louisiana.

In June, 1996, Dr. Shuler presented a talk at the Life Technologies, Inc. facility in Grand Island, New York. Both Dr. Gorfien and Mr. Godwin attended that talk. In that talk, Dr. Shuler discussed the use of dextran sulfate to culture insect cells.

IV. The Rejection Of Claims 1-29, 79, 83 and 140-142 Over Israel Must Be Withdrawn

At page 2 of the Office Action, the Examiner rejected claims 1-29, 79, 83 and 140-142, under 35 U.S.C. § 102(b), as allegedly anticipated by Israel, U.S. Patent Number 5,318,898 ("Israel"). Applicants respectfully traverse this rejection.

Claims 83, 141 and 142 have been canceled without prejudice to or disclaimer of the subject matter therein.

As amended, claims 1, 15 and 22 recite methods of cultivating a mammalian cell in suspension *in vitro*, using a medium that is chemically defined. Claims 2-14, 79, and 140 depend, either directly or indirectly, from claim 1. Claims 16-21 depend, either directly or indirectly, from claim 15. Claims 23-29 depend, either directly or indirectly, from claim 22.

Israel fails to anticipate claims 1-29, 79, and 140, because Israel fails to teach cultivation of a cell in suspension *in vitro*, using a medium that is chemically defined.

Israel also fails to anticipate claim 6, because Israel fails to teach a medium that is protein-free.

Israel also fails to anticipate claim 7, in which a 1X medium formulation is recited.

Israel also fails to anticipate claim 8, in which a 10X concentrated medium formulation is recited.

Israel also fails to anticipate claims 15, 20, 21 and 27-29, because Israel fails to teach the ingredients recited in claims 15, 20, 21 and 27-29.

Israel also fails to anticipate claim 140, because Israel fails to teach a medium that is free of animal-derived ingredients.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

V. The Rejection Of Claims 30-37 Over Israel and WO 92/05246 Must Be Withdrawn

At page 4 of the Office Action, the Examiner rejected claims 30-37, under 35 U.S.C. § 103(a), as allegedly obvious over Israel in view of Ramos *et al.*, WO 92/05246

("WO 92/05246").[1] Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Even in combination, Israel and WO 92/05246 would not have suggested the method of claims 30-37.

As amended, claims 1, 15 and 22 recite a method of cultivating a mammalian cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound. Claims 30-37 depend, either directly or indirectly, from any one of claims 1, 15 or 22.

Israel fails to teach cultivation of a cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound. WO 92/05246 fails to cure the deficiency of Israel. At page 4, lines 7-10, WO 92/05246 teaches that yeast hydrolysate be used. Yeast hydrolysate is a non-chemically defined mixture.

One of ordinary skill in the art would not have been motivated to combine Israel and WO 92/05246 in an effort to obtain a method of culturing cells in a chemically defined medium, because Israel and WO 92/05246 would have suggested neither a chemically defined medium, nor the benefits obtained by using a chemically defined medium. Moreover, even in

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[1] At page 4 of the Office Action, the Examiner stated that it is presumed that "the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary." As Applicants explained at page 10 of the First Supplemental Information Disclosure Statement filed September 24, 1999, Dr. McClure is a co-inventor of claims 1-83 and 139-142, and is an employee at Eli Lilly and Company. Dr. Gorfien is a co-inventor of claims 84-138 and 143-145, and is an employee at Life Technologies, Inc.

The inventions of claims 1-83 and 139-142 (McClure *et al.*) and 84-138 and 143-145 (Gorfien *et al.*) were not commonly owned at the time the McClure *et al.* and the Gorfien *et al.* inventions were made. Based on information available to the undersigned at the present time, the respective dates of invention of the McClure *et al.* and Gorfien *et al.* inventions cannot be determined at the present time.

combination, Israel and WO 92/05246 would not have provided any expectation of success in obtaining a chemically defined medium.

Even in combination, Israel and WO 92/05246 would not have suggested a method of cultivating cells using a serum-free, non-chemically defined medium. Therefore, Israel and WO 92/05246 would not have suggested the method of claims 30-37. Applicants respectfully request that this rejection be reconsidered and withdrawn.

VI. The Rejection Of Claims 73-83 Over Israel, WO 92/05246 and Inlow Must Be Withdrawn

At page 5 of the Office Action, the Examiner rejected claims 73-83, under 35 U.S.C. § 103(a), as allegedly obvious over Israel in view of WO 92/05246 and Inlow, U.S. Patent Number 5,024,947 ("Inlow"). Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Even in combination, Israel, WO 92/05246 and Inlow would not have suggested the method of claims 73-83.

Claims 78 and 83 have been canceled without prejudice to, or disclaimer of, the subject matter therein.

Claim 73 is directed to a method of producing a virus. Claims 73 and 79 depend multiply from any one of claims 1, 15 or 22. As amended, claims 1, 15 and 22 recite methods of cultivating a mammalian cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound. Claims 74-77 depend, either directly or indirectly, from claim 73. Claims 80-82 depend, either directly or indirectly, from claim 79.

Israel fails to teach cultivation of a cell in suspension *in vitro*, using a chemically defined cell culture medium comprising at least one polyanionic or polycationic compound. WO 92/05246 fails to cure the deficiency of Israel. At page 4, lines 7-10, WO 92/05246 teaches that yeast hydrolysate be used. Yeast hydrolysate is a non-chemically defined mixture. Likewise, Inlow fails to cure the deficiency of Israel and WO 92/05246, because Inlow fails to teach a chemically defined serum-free medium.

One of ordinary skill in the art would not have been motivated to combine Israel, WO 92/05246, and Inlow in an effort to obtain a method of culturing cells in a chemically defined medium, because Israel, WO 92/05246 and Inlow would have suggested neither a chemically defined medium, nor the benefits obtained from using a chemically defined medium.

Further, one of ordinary skill in the art would not have been motivated to combine Inlow with Israel and WO 92/05246, because Inlow relates only to the culture of insect cells, not mammalian cells. There is no assurance in Israel, WO 92/05246, or Inlow that a serum-free medium that facilitates production of virus in insect cells would facilitate the production of virus in mammalian cells.

Moreover, even in combination, Israel, WO 92/05246 and Inlow would not have provided any expectation of success in obtaining a serum-free, chemically defined medium that facilitates the production of virus in mammalian cells.

Even in combination, Israel, WO 92/05246 and Inlow would not have suggested a method of producing virus in mammalian cells using non-chemically defined medium. Therefore, Israel, WO 92/05246 and Inlow would not have suggested the method of claims 73-77 and 79-82. Applicants respectfully request that this rejection be reconsidered and withdrawn.

VII. The Rejection Of Claims 78 and 83 Over Inlow Must Be Withdrawn

At page 5 of the Office Action, the Examiner rejected claims 78 and 83, under 35 U.S.C. § 102(b), as allegedly anticipated by Inlow. Applicants respectfully traverse this rejection.

Claims 78 and 83 have been canceled without prejudice to, or disclaimer of, the subject matter therein. Accordingly, applicants respectfully request that this rejection be withdrawn.

VIII. The Rejection Of Claims 106 and 143-149 Over Keen Must Be Withdrawn

At page 6 of the Office Action, the Examiner rejected claims 106 and 143-149, under 35 U.S.C. § 102(b), as allegedly anticipated by Keen, U.S. Patent Number 5,316,938 ("Keen"). Applicants respectfully traverse this rejection.

As amended, claim 106 recites a method of cultivating mammalian cells in suspension culture and/or expressing a recombinant protein, using a cell culture medium comprising an iron chelate and a zinc salt, and that does not contain insulin. Claims 143-149 depend from claim 106.

Keen relates to a culture medium for culturing engineered CHO cells. Keen fails to anticipate claims 106 and 143-149, because Keen teaches that the medium *must* include a growth factor.

> The present invention therefore provides a biochemically defined culture medium for culturing engineered CHO cells . . . comprising water . . . and a recombinant or synthetic growth factor and *optionally* non-ferrous metal ions, vitamins and cofactors.

Keen at column 3, lines 2-11 (emphasis added). Had addition of a growth factor been optional, Keen would have indicated such, because Keen knew how to indicate whether addition of an ingredient is optional. *See* Keen at column 3, line 10.

Keen also teaches that such growth factors include insulin. *See* Keen at column 5, lines 36-43. Further, the only two media exemplified by Keen each contain insulin. *See* Keen at column 7, line 61; and column 8, line 23.

Keen also fails to teach the method of claim 143, because Keen fails to teach a medium that is free of animal-derived ingredients.

Keen also fails to teach the method of claim 144, because Keen fails to teach a medium that is protein-free.

Keen also fails to teach the method of claim 145, because Keen fails to teach a medium that is does not include insulin, and that is chemically defined.

Keen also fails to teach the method of claim 146, because Keen fails to teach a medium that contains neither transferrin nor insulin.

Keen also fails to teach the method of claim 147, because Keen fails to teach a medium that does not include insulin, and that supports the growth of CHO cells.

Keen also fails to teach the method of claim 148, because Keen fails to teach a 1X medium formulation that does not include insulin.

Keen also fails to teach the method of claim 149, because Keen fails to teach a concentrated medium formulation that does not include insulin.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

IX. The Rejection Of Claims 150-153 Over Keen Must Be Withdrawn

At page 6 of the Office Action, the Examiner rejected claims 150-153, under 35 U.S.C. § 103(a), as allegedly obvious over Keen. Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Keen teaches away from the claimed method.

Claims 150 and 151 depend from claim 149, and claim 149 depends from claim 106. Claims 152 and 152 depend, either directly or indirectly, from claim 106. As amended, claim 106 recites a method of cultivating mammalian cells in suspension culture and/or expressing a recombinant protein, using a cell culture medium comprising an iron chelate and a zinc salt, and that does not contain insulin.

At page 6 of the Office Action, the Examiner stated:

> Keen et al. teach various culture media formulations and the iron and zinc component ingredients to also be contained by these formulations. Thus, it would have been well within the purview of one of skill to formulate specific components for use in the culture medium of Keen et al. Further, the disclosure of Keen et al. would have motivated one of skill in the art to provide for these media. Therefore, the claims are prima facie obvious over the cited reference.

Applicants respectfully disagree. The standard for obviousness is *not* whether claimed subject matter is allegedly "within the purview" of the artisan. As the MPEP provides:

> A statement that modifications of the prior art to meet the claimed invention would have been "'well within the ordinary skill of the art'" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references.

MPEP § 2143.01, page 2100-99 (Feb. 1, 2000) (citation omitted). Here, the Examiner has done what the MPEP explicitly instructs should not be done. Thus, the basis for this rejection is improper.

The standard for obviousness is whether, absent Applicants' disclosure, the cited art (1) would have led the artisan to attempt to obtain the claimed invention, and (2) would have provided a reasonable expectation of success in obtaining the claimed invention.

For Keen to have provided motivation, Keen must have led one of ordinary skill in the art to obtain the claim invention. Here, and Keen would not have led one of ordinary skill in the art to the medium of the methods of claims 150-153, because Keen teaches explicitly that a medium must contain insulin. Thus, Keen teaches away from the claimed methods.

For Keen to have provided a reasonable expectation of success, Keen must have predicted that a medium devoid of insulin would have supported the growth of cells and/or the expression of recombinant protein. Given the explicit teaching in Keen to use insulin, Keen could not have provided the artisan with any expectation of success in obtaining a medium that does not contain insulin and that would have supported the growth of cells and/or the expression of recombinant protein.

Thus, Keen would not have suggested the method of claim 106. Since Keen would not suggested the method of claim 106, Keen would not have suggested the method of claim 150, in which a 10X medium formulation is recited, or the method of claim 151, in which a medium formulation of greater than 10X is recited.

Keen would also not have suggested the method of claim 152, because Keen would not have suggested the desirability of the specific range of iron and the specific range of zinc that are recited in claim 152.

Keen would also not have suggested the method of claim 153, because Keen would not have suggested the desirability of the specific concentration of iron and the specific concentration of zinc that are recited in claim 153.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

X. The Rejection Of Claims 106-112 and 143-153 Over Keen, Israel and Inlow Must Be Withdrawn

At page 6 of the Office Action, the Examiner rejected claims 106-112 and 143-153, under 35 U.S.C. § 103(a), as allegedly obvious over Keen in view of Israel and Inlow. Applicants respectfully traverse this rejection. A *prima facie* case of obviousness has not been established. Even in combination, Keen, Israel and Inlow would not have suggested the methods of claims 106-112 and 143-153

Claims 107-112 and 143-153 depend, either directly or indirectly, from claim 106. As amended, claim 106 recites a method of cultivating mammalian cells in suspension culture and/or expressing a recombinant protein, using a cell culture medium comprising an iron chelate and a zinc salt, and that does not contain insulin.

As discussed above, Keen teaches away from the claimed methods, because Keen teaches that insulin is necessary. Israel and Inlow fail to cure the deficiency of Keen, because neither Israel nor Inlow would not have suggested that a medium comprising an iron chelate and a zinc salt, and that does not contain insulin, would support cultivation of mammalian cells in suspension culture and/or expression of a recombinant protein. Further, Inlow relates only to insect cells, not to mammalian cells.

Moreover, Applicants have discovered that, in a chemically defined medium, transferrin can be replaced by an iron chelate, and insulin can be replaced by a zinc salt. Keen, Israel and Inlow would not have suggested either that transferrin can be replaced by an iron chelate, or that insulin can be replaced by a zinc salt.

Therefore, even in combination, Keen, Israel and Inlow would have taught away from the methods of claim 106-112 and 143-153.

Keen, Israel and Inlow would also not have suggested the method of claim 143, because Keen, Israel and Inlow would not have suggested a medium that is free of animal-derived ingredients.

Keen, Israel and Inlow would also not have suggested the method of claim 144, because Keen, Israel and Inlow would not have suggested a medium that is protein-free.

Keen, Israel and Inlow would also not have suggested the method of claim 145, because Keen, Israel and Inlow would not have suggested a medium that does not contain insulin and that is chemically defined.

Keen, Israel and Inlow would also not have suggested the method of claim 146, because Keen, Israel and Inlow would not have suggested a medium that contains neither transferrin nor insulin.

Keen, Israel and Inlow would also not have suggested the method of claim 147, because Keen, Israel and Inlow would not have suggested a medium that is does not include insulin, and that supports the growth of CHO cells.

Keen, Israel and Inlow would also not have suggested the method of claim 148, because Keen, Israel and Inlow would not have suggested a 1X medium formulation that does not include insulin.

Keen, Israel and Inlow would also not have suggested the method of claim 149, because Keen, Israel and Inlow would not have suggested a concentrated medium formulation that does not include insulin.

Keen, Israel and Inlow would also not have suggested the method of claim 150, in which a 10X medium formulation is recited, or the method of claim 151, in which a medium formulation of greater than 10X is recited.

Keen, Israel and Inlow would also not have suggested the method of claim 152, because Keen, Israel and Inlow would not have suggested the desirability of the specific range of iron and the specific range of zinc that are recited in claim 152.

Keen, Israel and Inlow would also not have suggested the method of claim 153, because Keen, Israel and Inlow would not have suggested the desirability of the specific concentration of iron and the specific concentration of zinc that are recited in claim 153.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

XI. Other Matters

A claim to priority under 35 U.S.C. § 119(a)-(d) and a certified copy of the priority document were filed on July 21, 2000. Prompt acknowledgment of this claim and submission is respectfully requested.

A Second Supplemental Information disclosure statement, a form PTO-1449 listing documents, and a copy of each listed document were filed on July 21, 2000. Consideration of the listed documents and making the same of record in the prosecution of the above-identified application is respectfully requested.

A Third Supplemental Information disclosure statement, a form PTO-1449 listing documents, and a copy of each listed document are being filed herewith. Consideration of the listed documents and making the same of record in the prosecution of the above-identified application is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed or rendered moot. Applicants therefore respectfully request that the Examiner reconsider and withdraw all of the presently outstanding rejections. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Date: 7/28/2000

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